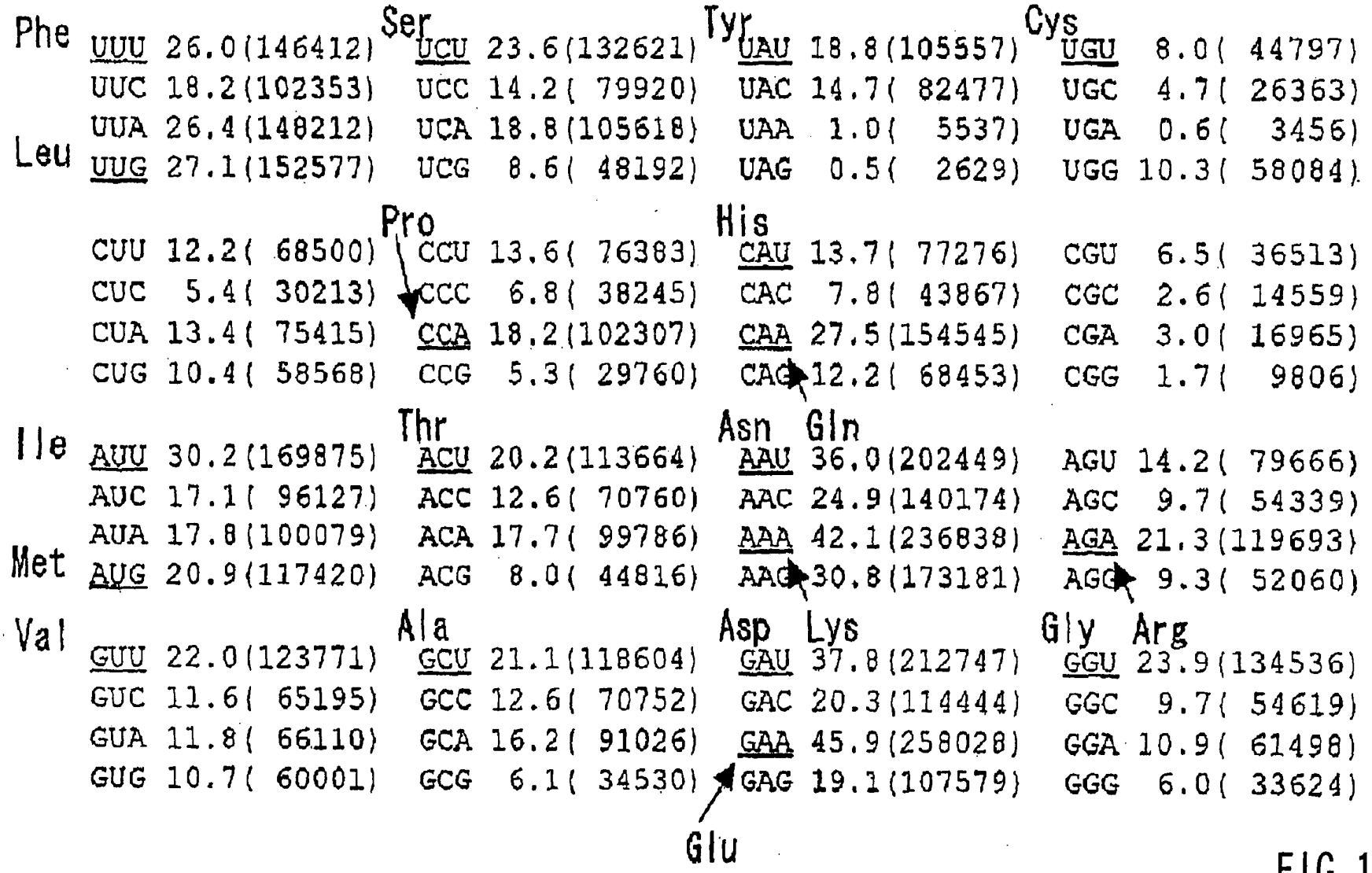


FIG. 1

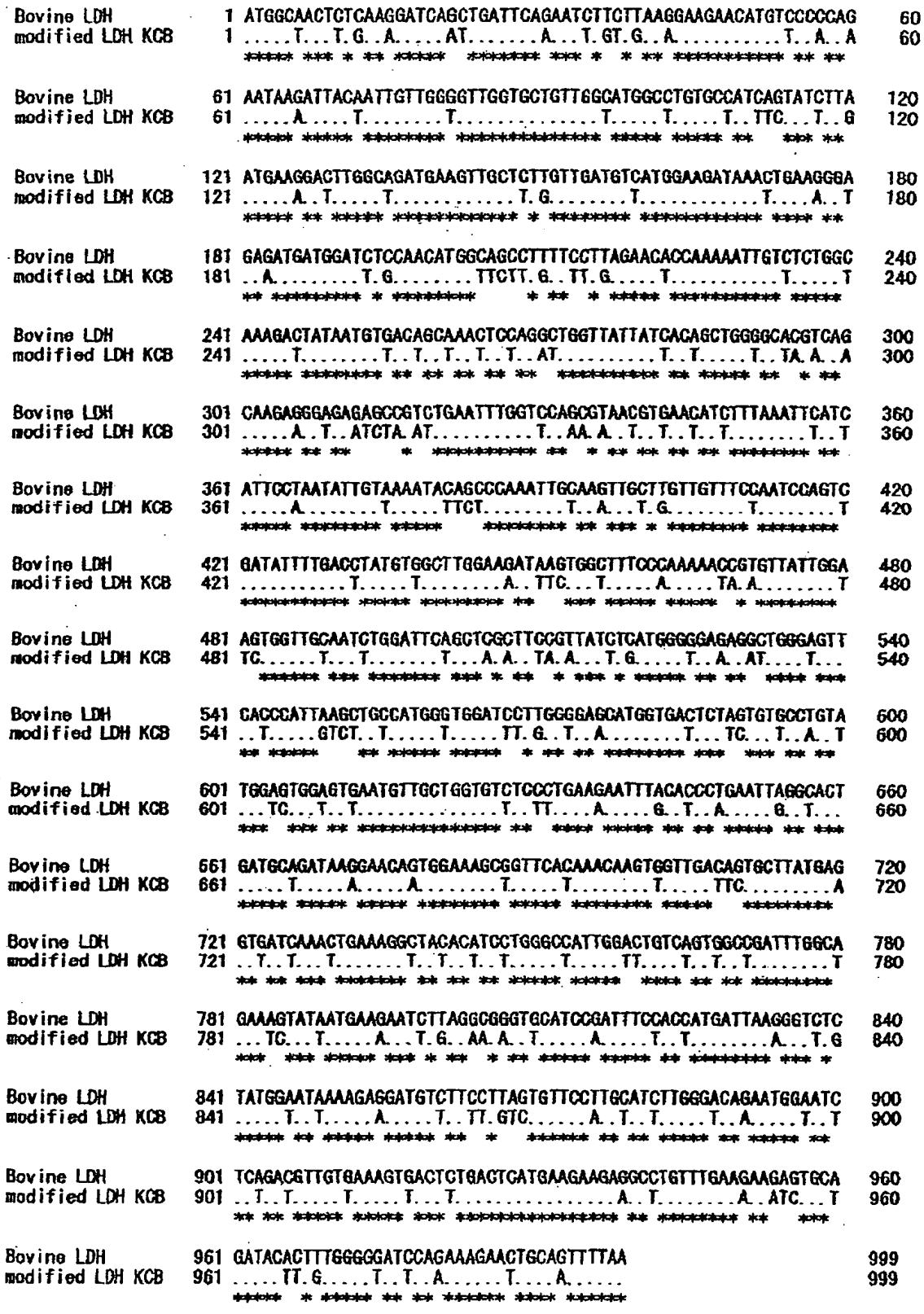

Bovine LDH 1 ATGGCAACTCTCAAGGATCAGCTGATTCAGAATCTTCTTAAGGAAGAACATGTCCCCCAG 60
modified LDH KCB 1 .....T...T.G..A.....AT.....A...T.GT.G..A.....T..A..A 60
Bovine LDH 61 AATAAGATTACAATTGTTGGGGTTGGTGCTGTTGGCATGGCCTGTGCCATCAGTATCTTA 120
modified LDH KCB 61 .....A.....T.....T.....T.....T.....T..TTC...T..G 120
Bovine LDH 121 ATGAAGGACTTGGCAGATGAAGTTGCTCTTGTTGATGTCATGGAAGATAAACTGAAGGGA 180
modified LDH KCB 121 .....A..T.....T.....T.G.....T.....T....A..T 180
Bovine LDH 181 GAGATGATGGATCTCCAACATGGCAGCCTTTTCCTTAGAACACCAAAAATTGTCTCTGGC 240
modified LDH KCB 181 ..A.....T.G.....TTCTT.G..TT.G.....T.....T.....T 240
Bovine LDH 241 AAAGACTATAATGTGACAGCAAACTCCAGGCTGGTTATTATCACAGCTGGGGCACGTCAG 300
modified LDH KCB 241 .....T.....T..T..T..T..T..AT.....T..T.....T..TA.A..A 300
Bovine LDH 301 CAAGAGGGAGAGAGCCGTCTGAATTTGGTCCAGCGTAACGTGAACATCTTTAAATTCATC 360
modified LDH KCB 301 .....A..T..ATCTA.AT.....T..AA.A..T..T..T..T.....T..T 360
Bovine LDH 361 ATTCCTAATATTGTAAAATACAGCCCAAATTGCAAGTTGCTTGTTGTTTCCAATCCAGTC 420
modified LDH KCB 361 .....A.....T.....TTCT.....T..A...T.G.....T.....T 420
Bovine LDH 421 GATATTTTGACCTATGTGGCTTGGAAGATAAGTGGCTTTCCCAAAAACCGTGTTATTGGA 480
modified LDH KCB 421 .....T.....T.....A..TTC...T.....A.....TA.A.....T 480
Bovine LDH 481 AGTGGTTGCAATCTGGATTCAGCTCGCTTCCGTTATCTCATGGGGGAGAGGCTGGGAGTT 540
modified LDH KCB 481 TC.....T...T.....T...A.A..TA.A...T.G.....T..A..AT....T... 540
Bovine LDH 541 CACCCATTAAGCTGCCATGGGTGGATCCTTGGGGAGCATGGTGACTCTAGTGTGCCTGTA 600
modified LDH KCB 541 ..T.....GTCT..T.....T.....TT.G..T..A.....T...TC...T..A..T 600
Bovine LDH 601 TGGAGTGGAGTGAATGTTGCTGGTGTCTCCCTGAAGAATTTACACCCTGAATTAGGCACT 660
modified LDH KCB 601 ...TC...T..T.....T..TT.....A.....G..T..A.....G..T... 660
Bovine LDH 661 GATGCAGATAAGGAACAGTGGAAAGCGGTTCACAAACAAGTGGTTGACAGTGCTTATGAG 720
modified LDH KCB 661 .....T.....A.....A.....T.....T.....T.....TTC.....A 720
Bovine LDH 721 GTGATCAAACTGAAAGGCTACACATCCTGGGCCATTGGACTGTCAGTGGCCGATTTGGCA 780
modified LDH KCB 721 ..T..T...T.....T..T..T..T.....T.....TT....T..T..T.....T 780
Bovine LDH 781 GAAAGTATAATGAAGAATCTTAGGCGGGTGCATCCGATTTCCACCATGATTAAGGGTCTC 840
modified LDH KCB 781 ...TC...T.....A...T.G..AA.A..T.....A.....T..T.....A...T.G 840
Bovine LDH 841 TATGGAATAAAAGAGGATGTCTTCCTTAGTGTTCCTTGCATCTTGGGACAGAATGGAATC 900
modified LDH KCB 841 .....T..T.....A.....T..TT.GTC.....A..T..T.....T..A.....T..T 900
Bovine LDH 901 TCAGACGTTGTGAAAGTGACTCTGACTCATGAAGAAGAGGCCTGTTTGAAGAAGAGTGCA 960
modified LDH KCB 901 ..T..T.....T.....T...T.....A..T.....A..ATC...T 960
Bovine LDH 961 GATACACTTTGGGGGATCCAGAAAGAACTGCAGTTTTAA 999
modified LDH KCB 961 .....TT.G.....T..T..A.....T....A..... 999

FIG. 2

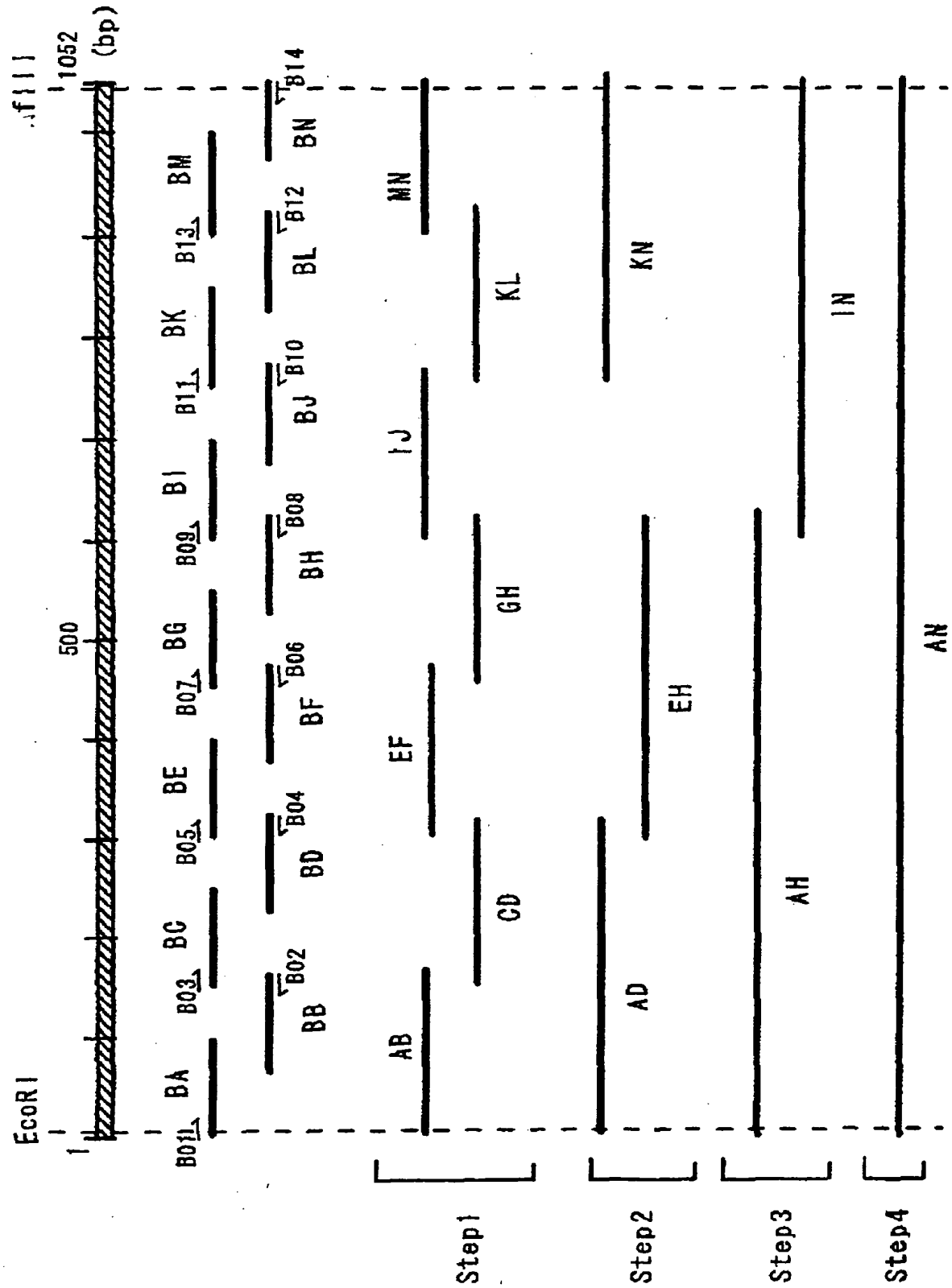
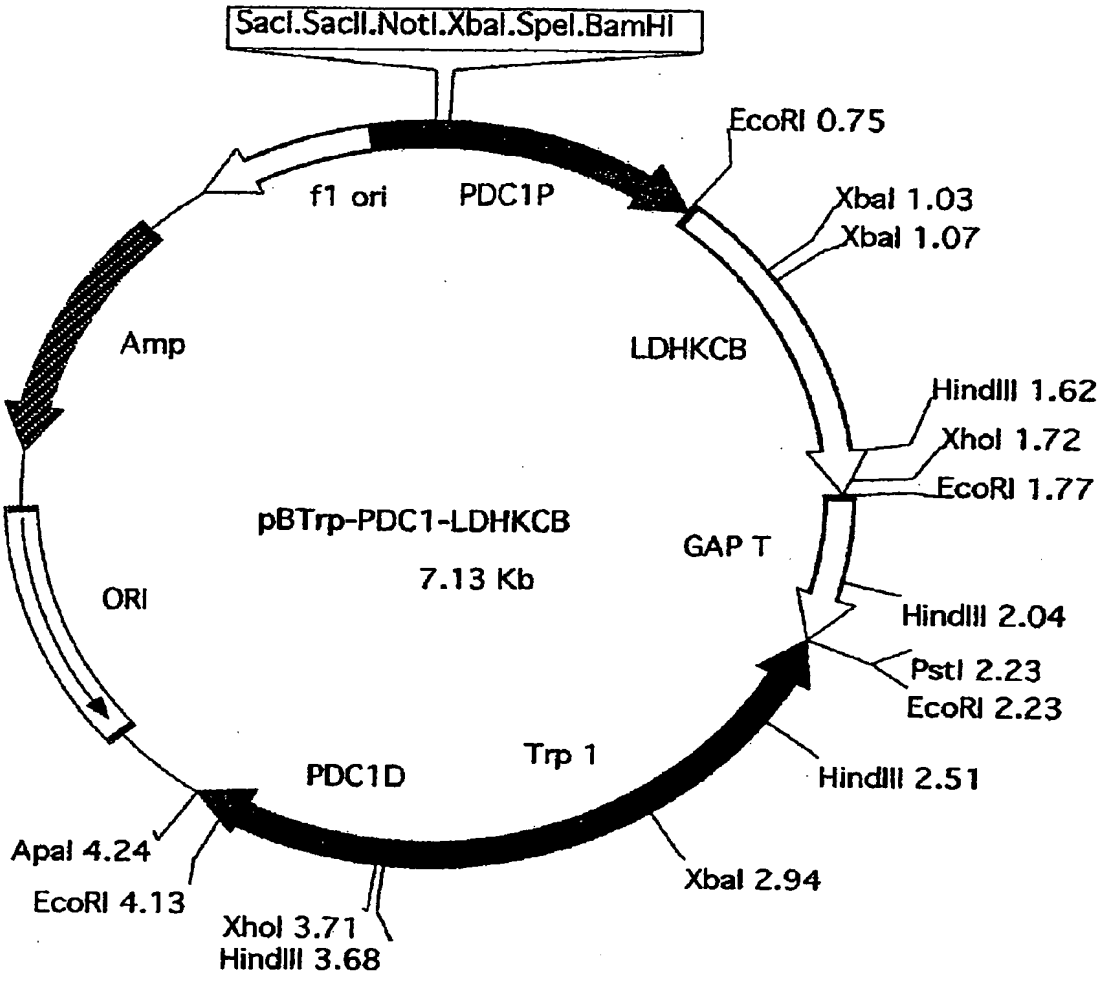

EcoRI
1
500
1052
(bp)
fill
B01
BA
B03
BC
B05
BE
B07
BG
B09
BI
B11
BK
B13
BM
B02
BB
B04
BD
B06
BF
B08
BH
B10
BJ
B12
BL
B14
BN
AB
EF
IJ
MN
CD
GH
KL
AD
EH
KN
AH
IN
AN
Step1
Step2
Step3
Step4

FIG. 3



Plasmid name: pBTrp-PDC1-LDHKCB
Plasmid size: 7.13 kb

FIG. 4

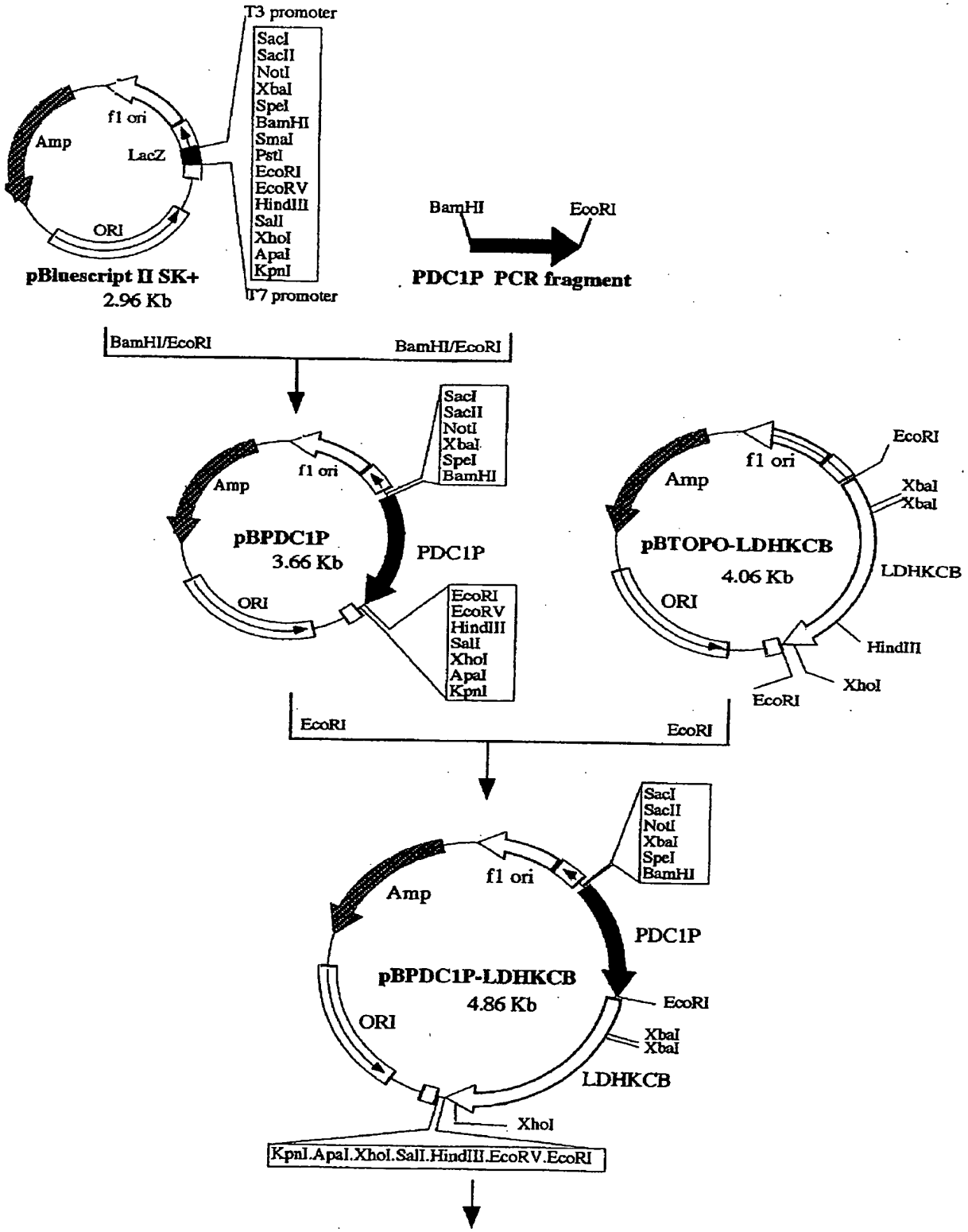

T3 promoter
SacI
SacII
NotI
XbaI
SpeI
BamHI
SmaI
PstI
EcoRI
EcoRV
HindIII
SalI
XhoI
ApaI
KpnI
T7 promoter
f1 ori
Amp
LacZ
ORI
pBluescript II SK+
2.96 Kb
BamHI
EcoRI
PDC1P PCR fragment
BamHI/EcoRI
BamHI/EcoRI
pBPDC1P
3.66 Kb
PDC1P
pBTOPO-LDHKCB
4.06 Kb
LDHKCB
HindIII
XhoI
EcoRI
EcoRI
EcoRI
pBPDC1P-LDHKCB
4.86 Kb
KpnI.ApaI.XhoI.SalI.HindIII.EcoRV.EcoRI

FIG. 5

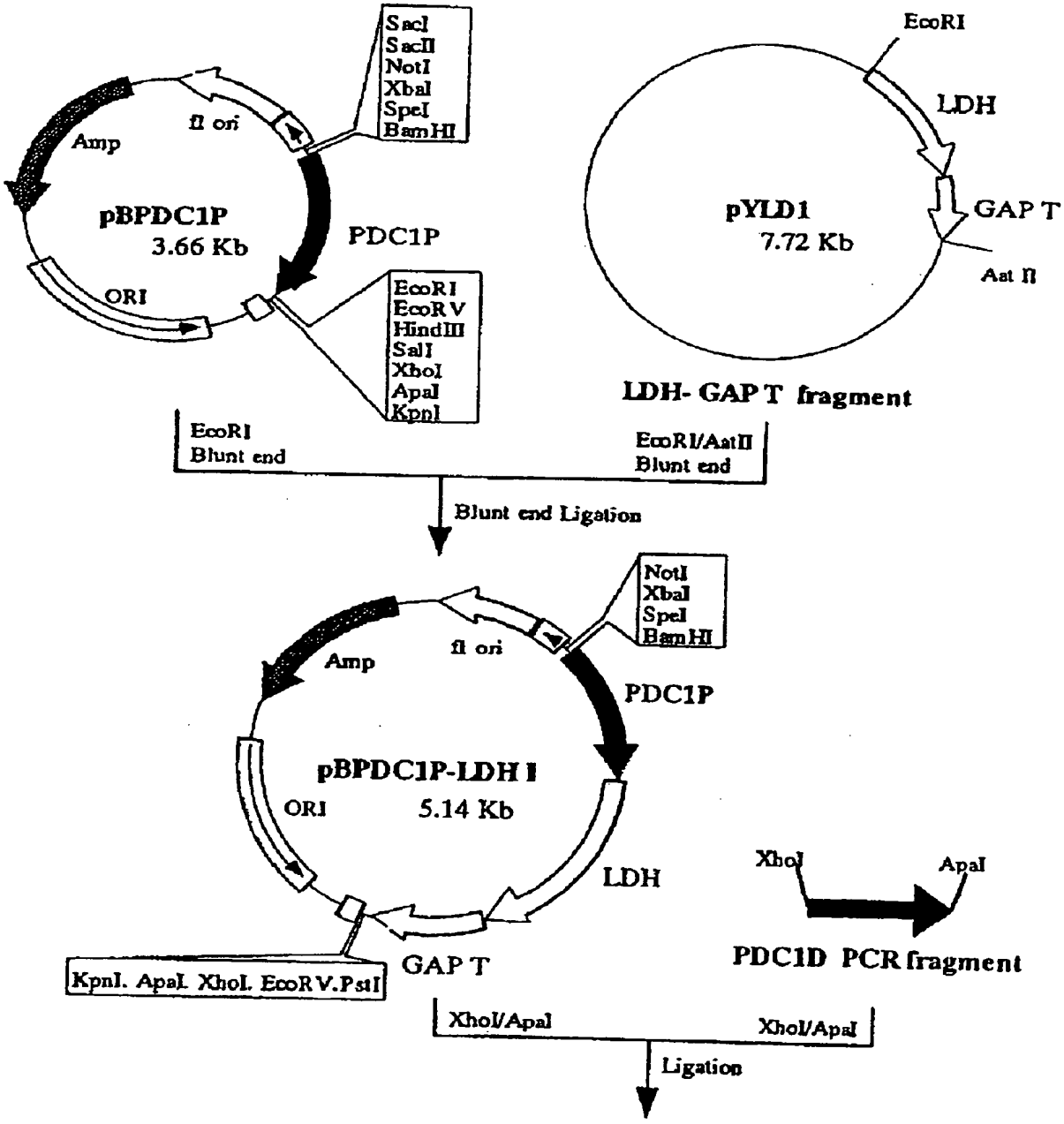

SacI
SacII
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
pBPDC1P
3.66 Kb
PDC1P
ORI
EcoRI
EcoRV
HindIII
SalI
XhoI
ApaI
KpnI
EcoRI
LDH
pYLD1
7.72 Kb
GAP T
Aat II
LDH- GAP T fragment
EcoRI
Blunt end
EcoRI/AatII
Blunt end
Blunt end Ligation
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
PDC1P
pBPDC1P-LDH I
ORI
5.14 Kb
LDH
GAP T
KpnI. ApaI. XhoI. EcoRV.PstI
XhoI
ApaI
PDC1D PCR fragment
XhoI/ApaI
XhoI/ApaI
Ligation

FIG. 6

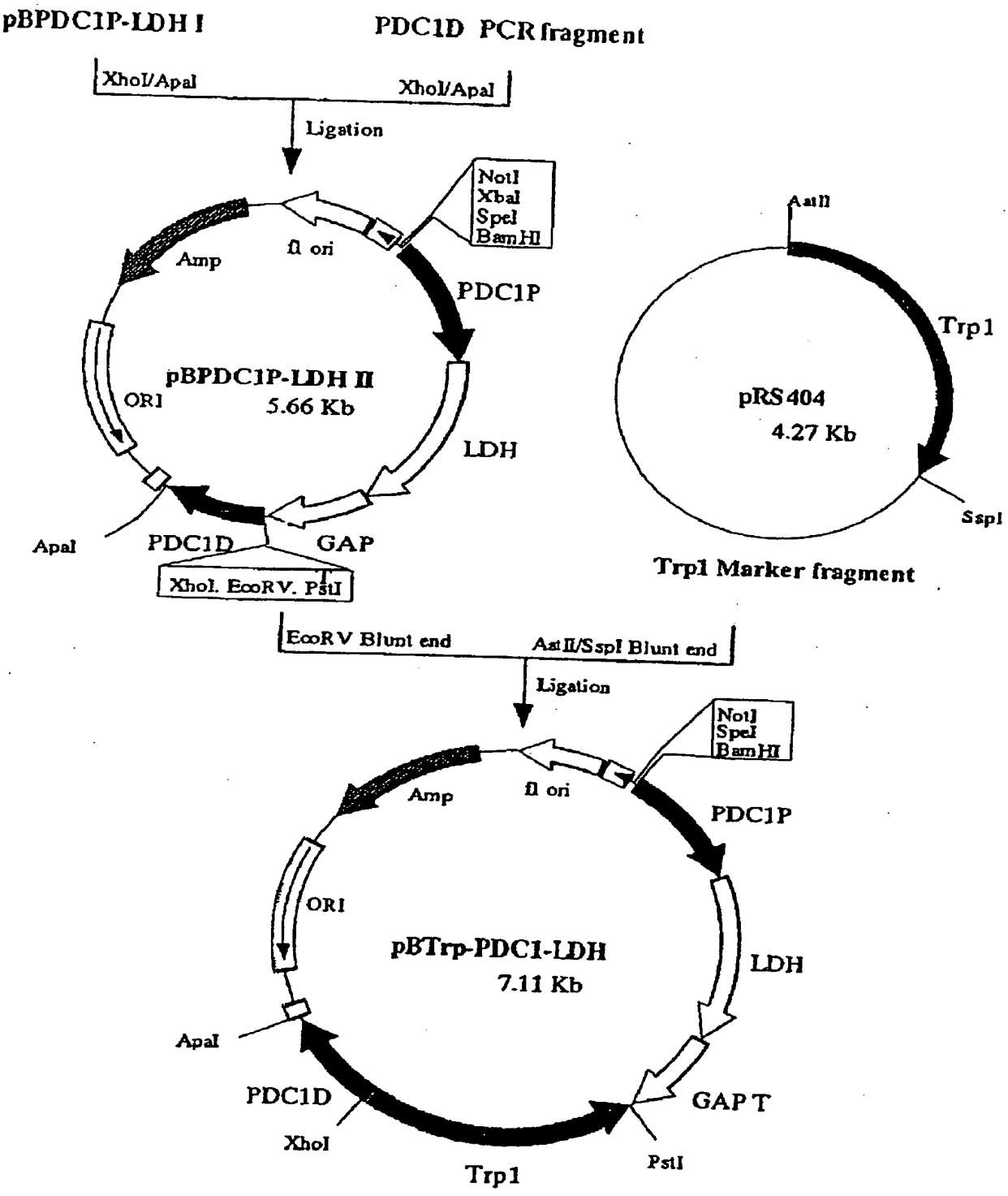

pBPDC1P-LDH I
PDC1D PCR fragment
XhoI/ApaI
XhoI/ApaI
Ligation
NotI
XbaI
SpeI
BamHI
Amp
fl ori
PDC1P
pBPDC1P-LDH II
5.66 Kb
ORI
LDH
ApaI
PDC1D
GAP
XhoI. EcoRV. PstI
AatII
Trp1
pRS404
4.27 Kb
SspI
Trp1 Marker fragment
EcoRV Blunt end
AatII/SspI Blunt end
Ligation
NotI
SpeI
BamHI
Amp
fl ori
PDC1P
ORI
pBTrp-PDC1-LDH
7.11 Kb
LDH
ApaI
PDC1D
GAP T
XhoI
Trp1
PstI

FIG. 7

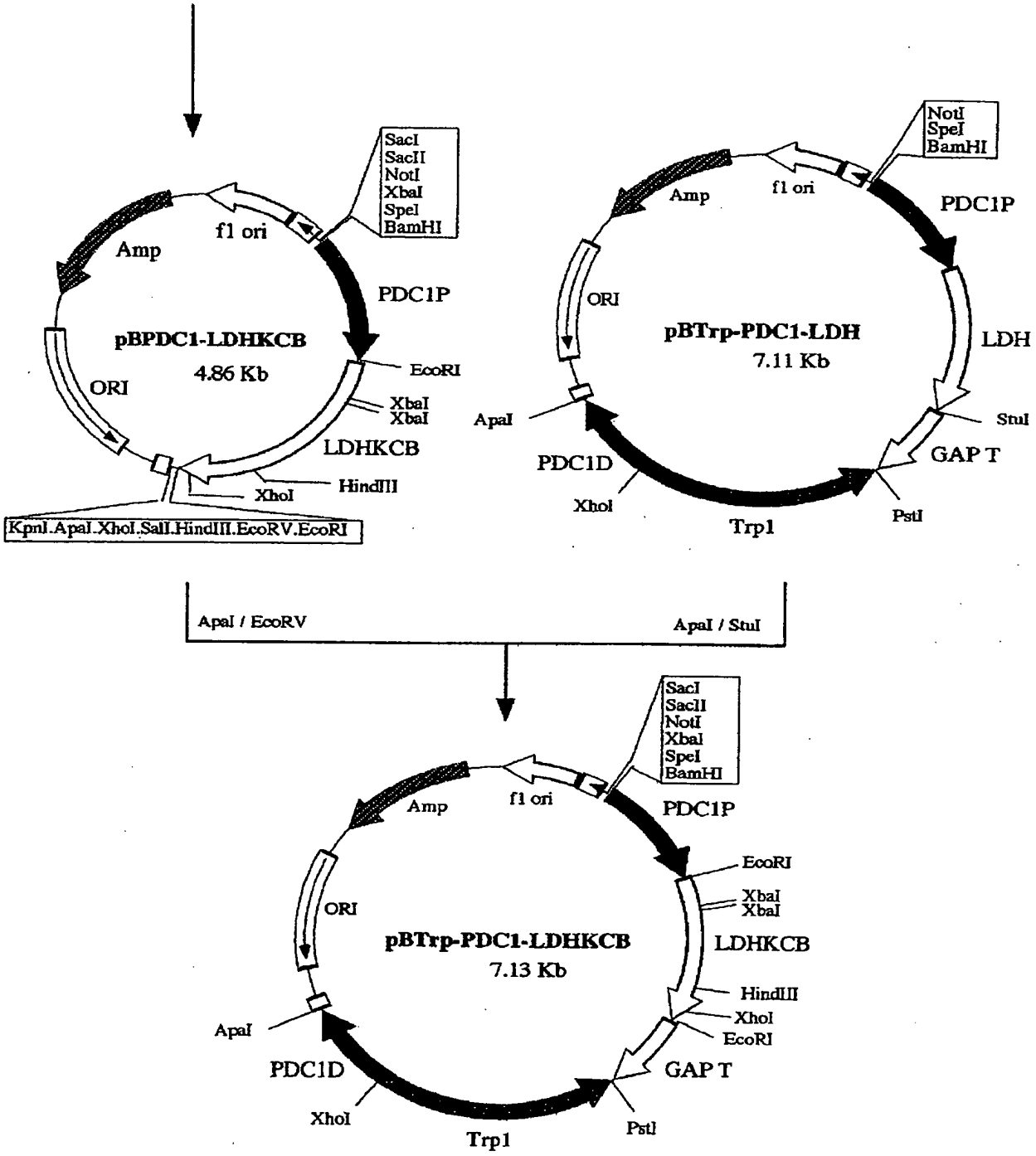

SacI
SacII
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
PDC1P
pBPDC1-LDHKCB
4.86 Kb
ORI
EcoRI
XbaI
XbaI
LDHKCB
HindIII
XhoI
KpnI.ApaI.XhoI.SalI.HindIII.EcoRV.EcoRI
NotI
SpeI
BamHI
f1 ori
Amp
PDC1P
ORI
pBTrp-PDC1-LDH
7.11 Kb
LDH
ApaI
StuI
PDC1D
GAP T
XhoI
PstI
Trp1
ApaI / EcoRV
ApaI / StuI
SacI
SacII
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
PDC1P
EcoRI
XbaI
XbaI
ORI
pBTrp-PDC1-LDHKCB
7.13 Kb
LDHKCB
HindIII
XhoI
EcoRI
ApaI
PDC1D
GAP T
XhoI
PstI
Trp1

FIG. 8

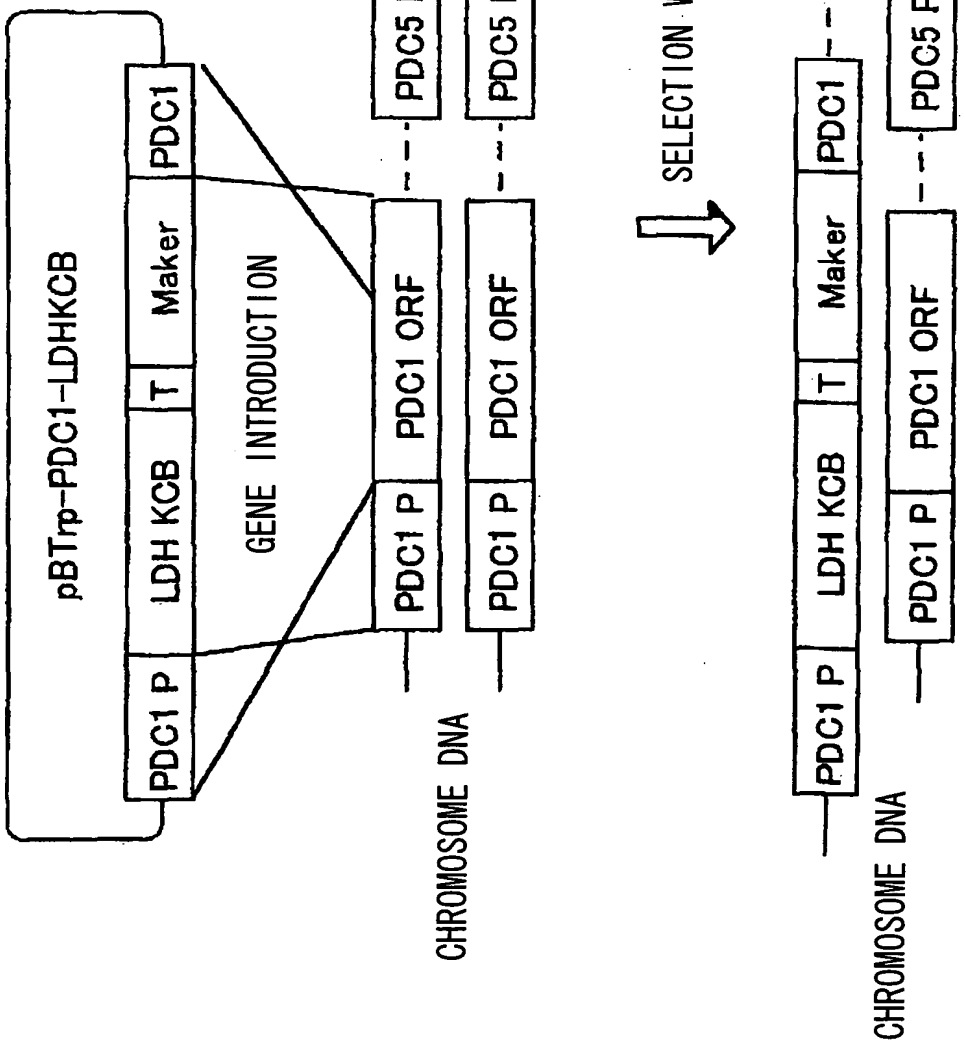

pBTrp-PDC1-LDHKCB
PDC1 P
LDH KCB
T
Maker
PDC1
GENE INTRODUCTION
PDC1 P
PDC1 ORF
PDC5 P
PDC5 ORF
CHROMOSOME DNA
SELECTION WITH TRP MAKER
PDC1 P
LDH KCB
T
Maker
PDC1
PDC5 P
PDC5 ORF
PDC1 P
PDC1 ORF
PDC5 P
PDC5 ORF
CHROMOSOME DNA

FIG. 9

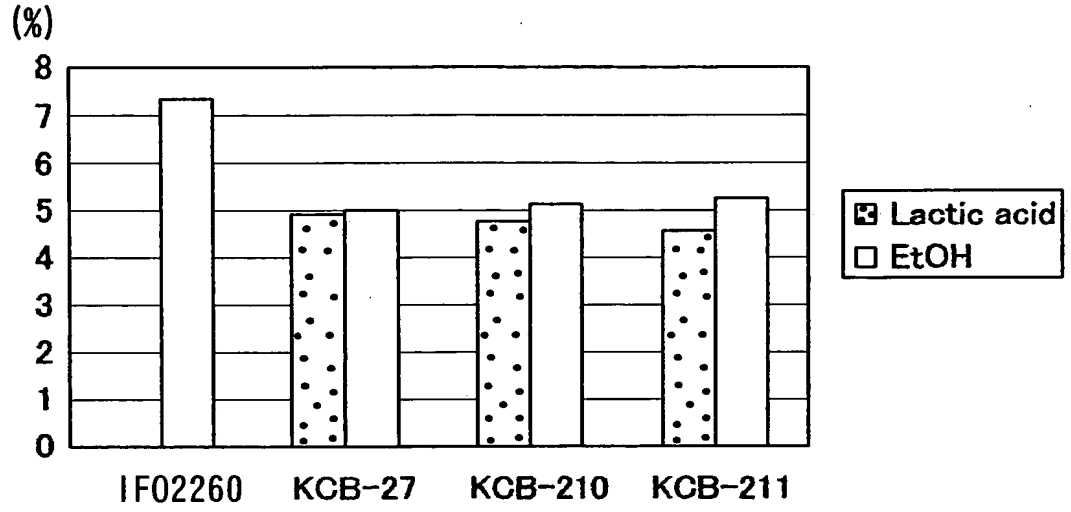


FIG. 10

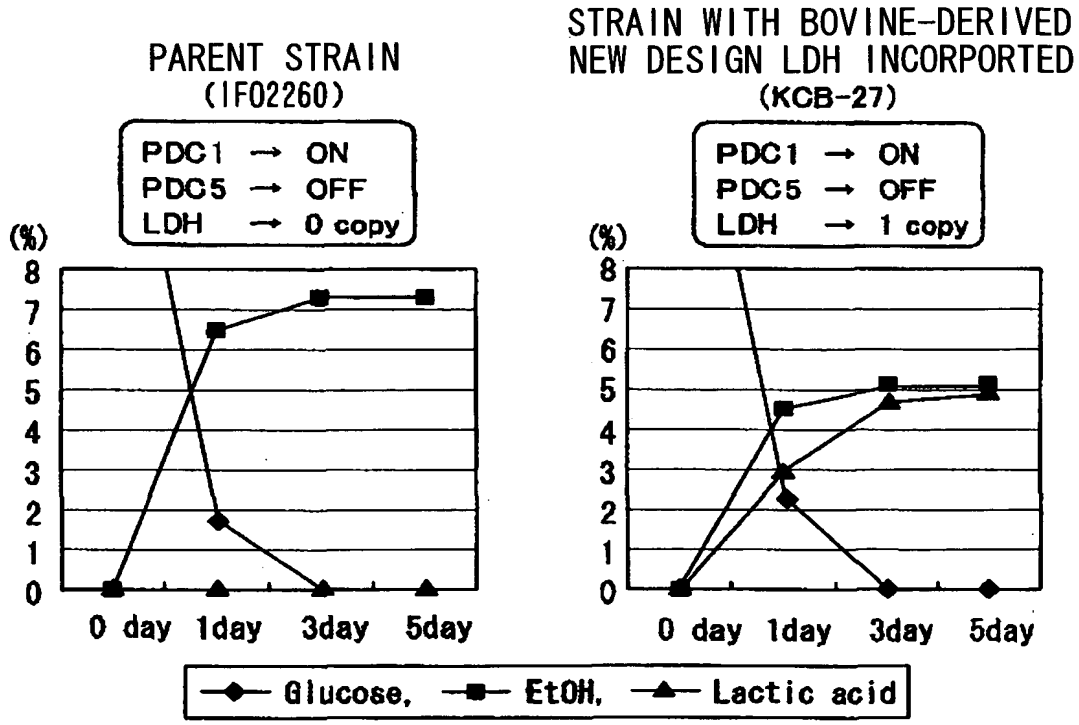

PARENT STRAIN
(IFO2260)
PDC1 → ON
PDC5 → OFF
LDH → 0 copy
(%)
8
7
6
5
4
3
2
1
0
0 day
1day
3day
5day
STRAIN WITH BOVINE-DERIVED
NEW DESIGN LDH INCORPORTED
(KCB-27)
PDC1 → ON
PDC5 → OFF
LDH → 1 copy
(%)
8
7
6
5
4
3
2
1
0
0 day
1day
3day
5day
Glucose,
EtOH,
Lactic acid

FIG. 11

BOVINE-MUSCLE-DERIVED
L-LACTATE DEHYDROGENASE (SIGMA) → Km=0.1mM

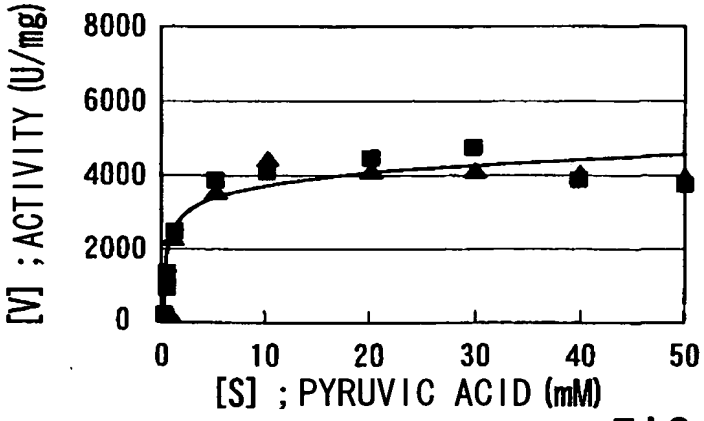


FIG. 12

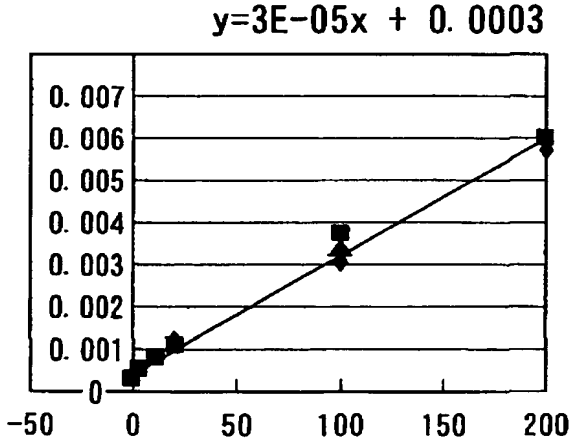


FIG. 13

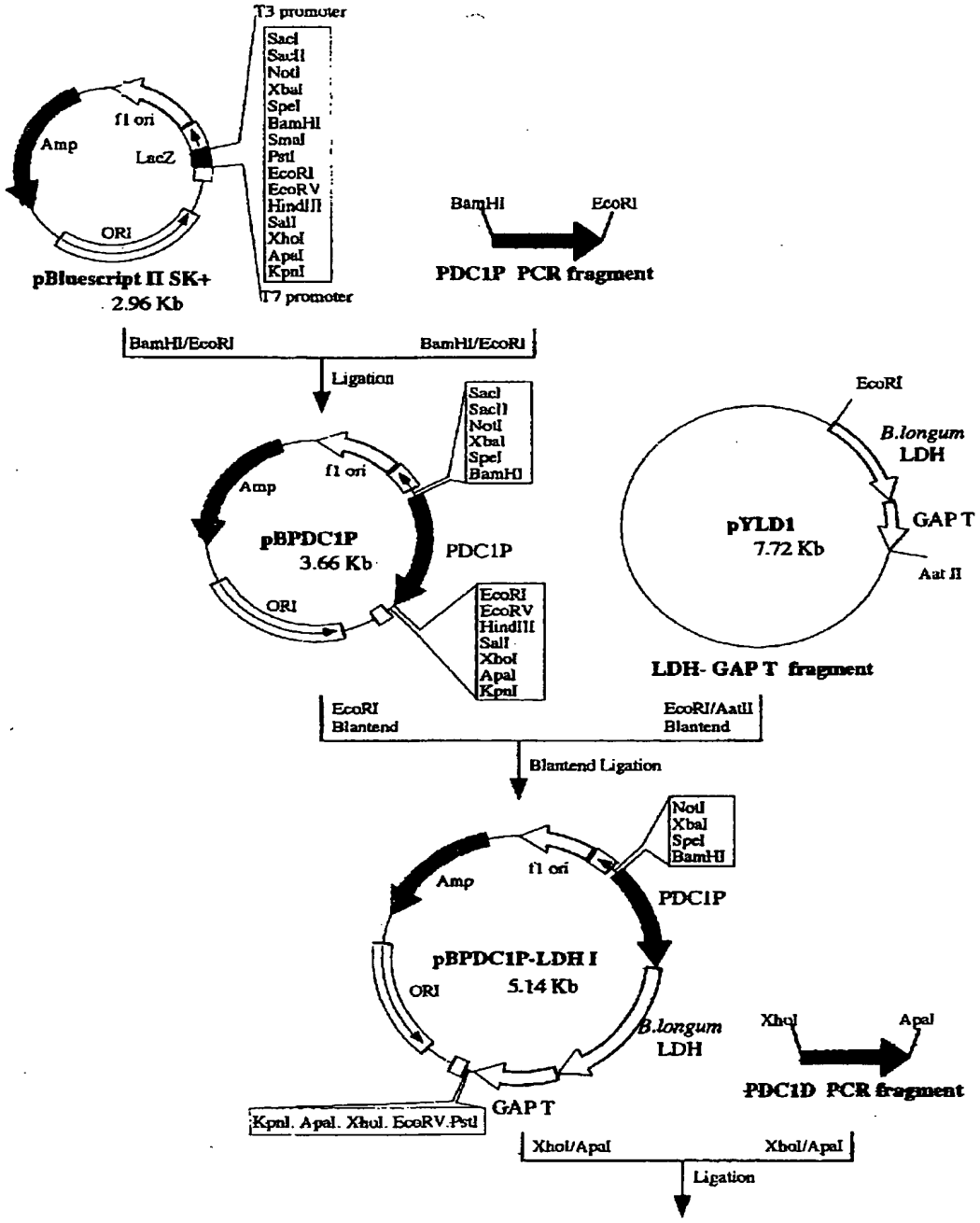

T3 promoter
SacI
SacII
NotI
XbaI
SpeI
BamHI
SmaI
PstI
EcoRI
EcoRV
HindIII
SalI
XhoI
ApaI
KpnI
T7 promoter
f1 ori
Amp
LacZ
ORI
pBluescript II SK+
2.96 Kb
BamHI
EcoRI
PDC1P PCR fragment
BamHI/EcoRI
BamHI/EcoRI
Ligation
SacI
SacII
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
pBPDC1P
3.66 Kb
PDC1P
ORI
EcoRI
EcoRV
HindIII
SalI
XhoI
ApaI
KpnI
EcoRI
B.longum
LDH
pYLD1
7.72 Kb
GAP T
Aat II
LDH- GAP T fragment
EcoRI
Blantend
EcoRI/AatII
Blantend
Blantend Ligation
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
PDC1P
pBPDC1P-LDH I
ORI
5.14 Kb
B.longum
LDH
XhoI
ApaI
PDC1D PCR fragment
GAP T
KpnI. ApaI. XhoI. EcoRV.PstI
XhoI/ApaI
XhoI/ApaI
Ligation

FIG. 14

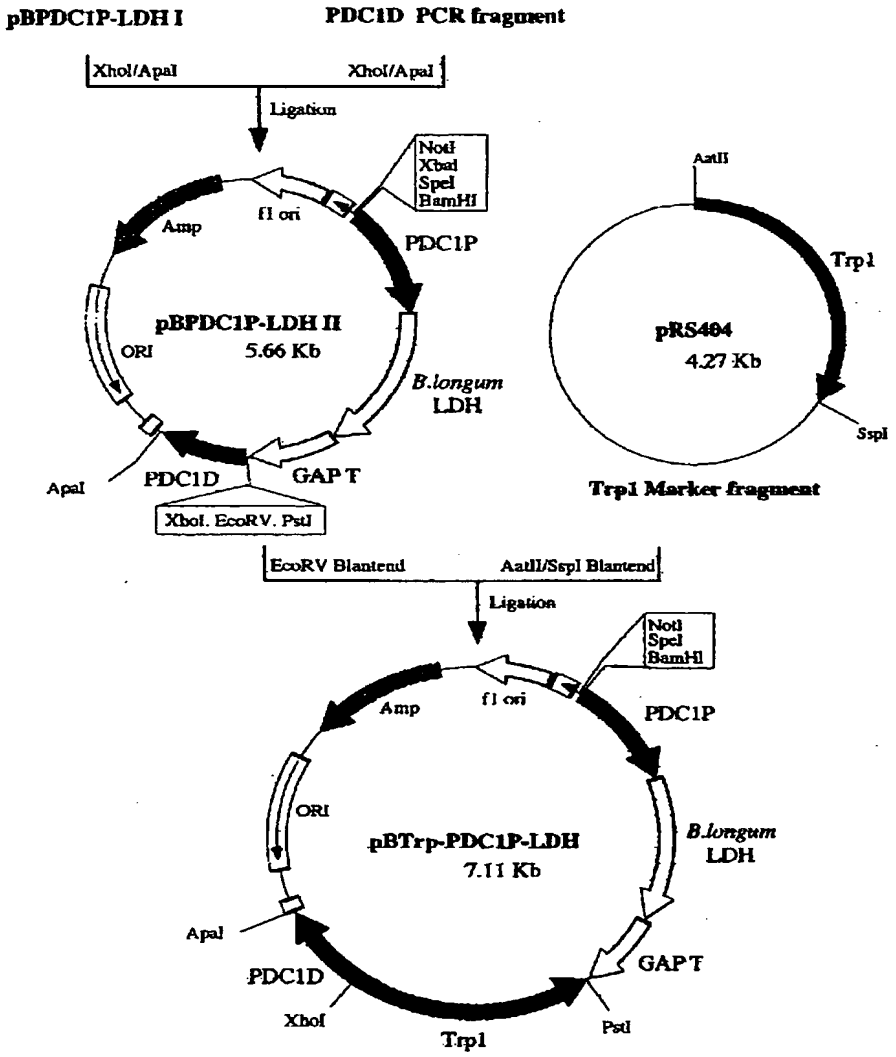

pBPDC1P-LDH I
PDC1D PCR fragment
XhoI/ApaI
XhoI/ApaI
Ligation
NotI
XbaI
SpeI
BamHI
f1 ori
Amp
PDC1P
pBPDC1P-LDH II
5.66 Kb
ORI
B.longum
LDH
ApaI
PDC1D
GAP T
XhoI. EcoRV. PstI
AatII
Trp1
pRS404
4.27 Kb
SspI
Trp1 Marker fragment
EcoRV Blantend
AatII/SspI Blantend
Ligation
NotI
SpeI
BamHI
f1 ori
Amp
PDC1P
ORI
pBTrp-PDC1P-LDH
7.11 Kb
B.longum
LDH
ApaI
PDC1D
GAP T
XhoI
Trp1
PstI

FIG. 15

YEAST-DERIVED PYRUVATE DECARBOXYLASE (SIGMA) → Km=0.346mM

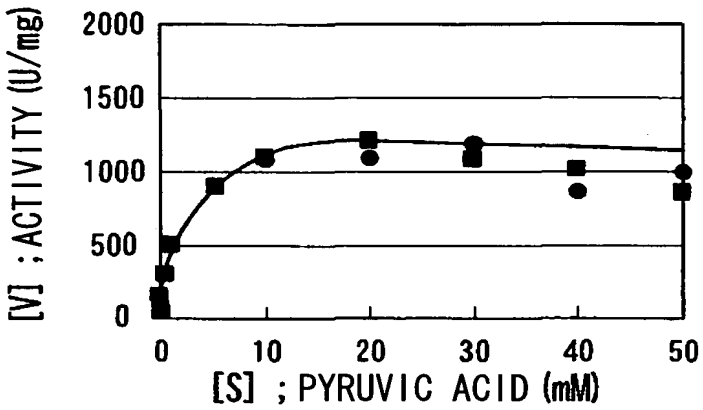


FIG. 16

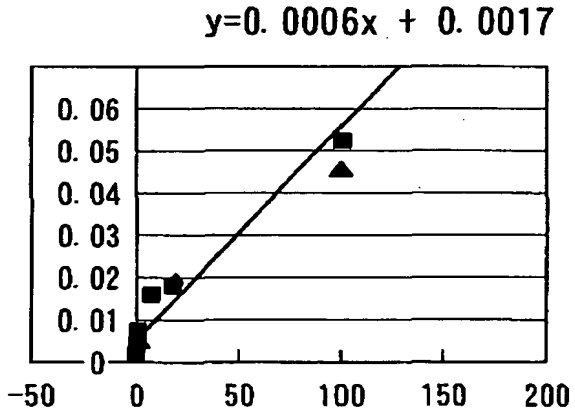


FIG. 17